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PREVALENCE AND ANTIMICROBIAL RESISTANCE PATTERNS OF ENTEROCOCCUS SPECIES IN CLINICAL SAMPLES.

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ABSTRACT **Background:** *Enterococcus* species are emerging nosocomial pathogens with increasing antimicrobial resistance, particularly to vancomycin. This study evaluates the prevalence, species distribution, and antimicrobial resistance patterns of *Enterococcus* isolates from clinical samples. **Methods:** An analysis of 150 *Enterococcus* isolates from urine, pus, blood, and body fluids was conducted. Species identification and antimicrobial susceptibility testing were performed using standard microbiological methods, following CLSI guidelines. **Results:** *E. faecalis* was the predominant species, followed by *E. faecium*. Urine samples were the most common source. Multi-drug resistance (MDR) was observed in a significant proportion of isolates, with high resistance to fluoroquinolones and aminoglycosides. Vancomycin-resistant *Enterococcus* (VRE) prevalence was 9.3%, more common in *E. faecium*. Linezolid and teicoplanin remained effective. **Conclusion:** The rising VRE and MDR *Enterococcus* burden highlights the need for surveillance, infection control, and antimicrobial stewardship programs to prevent further resistance spread.

KEYWORDS : Enterococcus, VRE, antimicrobial resistance, multi-drug resistance, clinical isolates, infection control

INTRODUCTION

Enterococcus species, once considered commensal organisms, have emerged as significant nosocomial pathogens due to their increasing resistance to multiple antibiotics. These bacteria are responsible for a wide range of infections, including urinary tract infections, bacteremia, surgical wound infections, and endocarditis.(Miller et al., 2014) Among clinically relevant species, *Enterococcus faecalis* and *Enterococcus faecium* account for the majority of infections, with their prevalence varying across different hospital settings.(Treitman et al., 2005)

A key concern in enterococcal infections is their ability to develop resistance to multiple antibiotics, including aminoglycosides, beta-lactams, and glycopeptides. The emergence of vancomycin-resistant *Enterococcus* (VRE) has further complicated treatment options, with resistance rates varying significantly across different regions. (Khandelwal et al., 2020) A study in a tertiary care hospital in India reported vancomycin resistance in 11.13% of *Enterococcus* isolates, whereas another study in Gujarat found a 14.09% prevalence of VRE, highlighting geographical variation in antimicrobial resistance.(Patel et al., 2020; Naik et al., 2016)

Recent studies in India have highlighted a shift in the epidemiology of *Enterococcus* infections, with an increasing prevalence of *E. faecium*, which is often more resistant to antibiotics than *E. faecalis*.(Smout et al., 2023) The ability of *Enterococcus* species to acquire and transfer resistance genes, including those encoding high-level aminoglycoside resistance and vancomycin resistance, makes them a critical challenge in healthcare settings.(Mittal et al., 2016) The rising incidence of multidrug-resistant *Enterococcus* necessitates enhanced infection control measures, routine antimicrobial susceptibility testing, and improved treatment strategies to prevent therapeutic failures. This study aims to contribute to the growing body of knowledge by providing updated data on the prevalence and antimicrobial resistance patterns of *Enterococcus* species in a hospital setting.

Methodology

Study Design And Setting

This observational Study was conducted at a Index Medical College Hospital and Research Centre in Indore, Madhya Pradesh, India, over a period of 18 months, analyzing 150 non-repetitive clinical isolates of *Enterococcus* species obtained from various clinical specimens, including urine, blood, pus, and body fluids. The study aimed to determine the prevalence, species distribution, and antimicrobial resistance patterns of *Enterococcus* isolates, with a focus on vancomycin resistance and multi-drug resistance (MDR).

Inclusion Criteria:

- Pure isolates of *Enterococcus* from clinical specimens.
- Samples from both inpatients and outpatients.

Exclusion Criteria:

- Mixed bacterial growth.
- Contaminated or inadequately labeled samples.

Bacterial Isolation And Identification

All clinical specimens were inoculated on Blood agar, MacConkey agar, and Chromogenic agar and incubated at 37°C for 24 hours. The isolates were identified as *Enterococcus* based on their Gram-positive cocci morphology in pairs or short chains, along with standard biochemical tests, including the catalase test, bile esculin test, growth in 6.5% NaCl, and growth at 45°C. Species-level identification was performed using standard biochemical reactions.

Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar as per Clinical and Laboratory Standards Institute (CLSI) guidelines. The following antibiotics were tested:

- Beta-lactams: Ampicillin, Penicillin
- Aminoglycosides: High-level gentamicin (120 µg), High-level streptomycin (300 µg)
- Glycopeptides: Vancomycin, Teicoplanin
- Fluoroquinolones: Ciprofloxacin
- Tetracyclines: Tetracycline
- Oxazolidinones: Linezolid

Vancomycin Resistance Detection:

Vancomycin resistance was detected using the disc diffusion method, where isolates were screened using a 30 µg vancomycin disc. Further confirmation was done using Minimum Inhibitory Concentration (MIC) testing with the E-test method, and the results were interpreted according to CLSI guidelines.

Multi-Drug Resistance (MDR) Definition & Quality Control Measures

Multi-drug resistance (MDR) was defined as resistance to three or more antibiotic classes, indicating the severity of antimicrobial resistance among *Enterococcus* isolates. To ensure accuracy, *Enterococcus faecalis* ATCC 29212 was used as a quality control strain for antimicrobial susceptibility testing. All culture media and antibiotic discs were procured from HiMedia (India) and used as per standard microbiological procedures.

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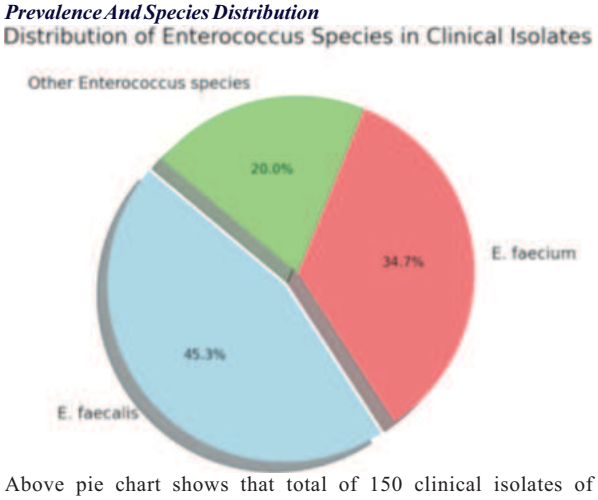
RESULT & DISCUSSION

Prevalence And Species Distribution



Above pie chart shows that total of 150 clinical isolates of *Enterococcus* species were obtained from different clinical specimens. Among these, *E. faecalis* accounted for 45.3% (n=68), *E. faecium* 34.7% (n=52), and other *Enterococcus* species 20.0% (n=30).

Table 1. Distribution of *Enterococcus* Isolates Across Clinical Samples n=150

Clinical Sample	Number of Isolates	Percentage (%)
Urine	76	50.9%
Pus	32	21.8%
Blood	24	16.4%
Body Fluids	18	10.9%

The highest number of isolates were from urine samples (50.9%), followed by pus (21.8%), blood (16.4%), and body fluids (10.9%), consistent with previous reports.

Table 2: Age And Gender Distribution Of *Enterococcus* Infections n=150

Age Group (Years)	Male	Female	Total (%)
0-10	2	1	3 (2.7%)
11-20	8	5	13 (11.8%)
21-30	9	4	13 (11.8%)
31-40	28	10	38 (34.5%)
41-50	10	4	14 (12.7%)
51-60	12	3	15 (13.6%)
61-70	10	4	14 (12.7%)
71-80	6	4	10 (9.1%)

Enterococcus infections were more frequently isolated from male patients (68.2%) compared to female patients (31.8%), with the highest occurrence in the 35–40 years age group (34.5%), aligning with previous studies.

Table 3: Antimicrobial Susceptibility Pattern of *Enterococcus* Species

Antibiotic	Susceptible (%)	Resistant (%)
Ampicillin	39.2%	60.8%
Penicillin	2.5%	97.5%
Ciprofloxacin	30.0%	70.0%
High-level Gentamicin	63.6%	36.4%
Tetracycline	19.1%	80.9%
Vancomycin	90.7%	9.3%
Linezolid	100.0%	0.0%
Teicoplanin	100.0%	0.0%

Table 4: Vancomycin Resistance Among *Enterococcus* Species (Final Correction)

Species	Total Isolates	VRE (%)	VRE Count
E. faecalis	68	5.5%	4 isolates
E. faecium	52	19.2%	10 isolates
Overall VRE Prevalence	150	9.3%	14 isolates

Table 5: Multi-Drug Resistance (MDR) in *Enterococcus* Isolates (Final Correction)

Resistance Pattern	Number of Isolates	Percentage (%)
Resistant to ≥3 Antibiotics	79	52.4%
Resistant to ≥5 Antibiotics	43	28.7%
MDR Including Vancomycin (VRE)	14	9.3%

The present study highlights the increasing burden of Enterococcus infections in a tertiary care hospital in India. E. faecalis was identified as the predominant species, followed by E. faecium, a trend consistent with previous hospital-based studies by Mohanty et al. (2005). (Mohanty et al., 2005) The highest number of isolates in this study were obtained from urine samples, supporting findings that Enterococcus is a major cause of urinary tract infections (UTIs) and catheter-associated infections.(Goel et al., 2016)

One of the significant concerns in this study was vancomycin resistance, which was more frequent in E. faecium (19.2%) compared to E. faecalis (5.5%). This finding aligns with research conducted by Raj et al. (2019) which observed increasing rates of vancomycin-resistant Enterococcus (VRE).(Raj et al., 2019) Studies have also indicated that E. faecium has a greater propensity for acquiring vancomycin resistance genes, making it a more challenging pathogen to treat.(Tuhina et al., 2016) Multi-drug resistance (MDR) was prevalent among the Enterococcus isolates in this study, with resistance rates against fluoroquinolones, aminoglycosides, and beta-lactams. Similar findings were reported in a study conducted Deshpande et al. (2013), where Enterococcus isolates exhibited high resistance to gentamicin and ciprofloxacin.(Deshpande et al., 2013) Another study from Rajasthan also confirmed high rates of MDR strains, including resistance to vancomycin and gentamicin.(Mathur, 2016)

The study results also indicate a shift in the epidemiology of Enterococcus infections, with E. faecium emerging as a dominant species, particularly in bloodstream infections. This trend has been observed in multiple Indian studies, suggesting a transition from E. faecalis to E. faecium dominance, which is concerning due to the latter's higher resistance rates.(Manavalan et al., 2015) E. faecium is more likely to develop resistance to linezolid and teicoplanin, making treatment options limited.(Mukherjee et al., 2016) The increasing prevalence of E. faecium in Indian hospitals reinforces the need for species-specific antimicrobial policies. The study underscores the urgent need for antimicrobial stewardship programs to prevent the

further spread of resistance. Surveillance and rational antibiotic use should be emphasized to mitigate the spread of MDR Enterococcus.(Sahu et al., 2015)

The findings from both this study and previous Indian research emphasize the alarming increase in vancomycin-resistant and multi-drug-resistant Enterococcus strains. While linezolid and teicoplanin remain effective, proactive infection control and antimicrobial stewardship measures are essential to prevent further resistance development in Indian healthcare settings.Our study underscores the rising threat of antimicrobial resistance in *Enterococcus* species, particularly VRE and MDR strains. While linezolid and teicoplanin remain effective treatment options, proactive measures must be taken to prevent further resistance development. Future research should focus on genotypic resistance mechanisms and the impact of antimicrobial stewardship programs in reducing resistance rates.

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